Ether Lipids of Planktonic Archaea in the Marine Water Column†

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Acyclic and cyclic biphytanes derived from the membrane ether lipids of archaea were found in water column particulate and sedimentary organic matter from several oxic and anoxic marine environments. Compound-specific isotope analyses of the carbon skeletons suggest that planktonic archaea utilize an isotopically heavy carbon source such as algal carbohydrates and proteins or dissolved bicarbonate. Due to their high preservation potential, these lipids provide a fossil record of planktonic archaea and suggest that they have thrived in marine environments for more than 50 million years.

The archaea belong to one of the three main groups of organisms on Earth (35). Until recently, archaea were thought to inhabit only ecological niches characterized by extreme conditions such as high salinity, high temperature, or anoxic environments (2). However, analyses of rRNA have shown that significant fractions (up to 34%) of marine bacterioplankton are archaea (4, 5, 11, 12). Their rRNAs do not resemble those of known archaea; therefore, planktonic archaea may constitute an important part of the planktonic community in coastal areas and open oceans. It is still not very clear, however, how widespread these organisms are, what their carbon and energy sources are, and what their evolutionary origin is.

A unique feature of archaea is their membrane lipids, which are derived from C_{20}-isopranyl diether and its tetraether dimer (18) and are readily distinguished from the acyl-ester membrane lipids of eubacteria and eukaryotes. These lipids are resistant to (bio)degradation and are thus readily preserved in sediments throughout geological history (18). Ether lipids have been used to detect archaea in hypersaline and anoxic environments (10, 24). The ether lipids reported in organisms and sediments can be divided into two main groups: one without rings and one with cyclopentane rings. The cyclized ether lipids have, so far, been found only in extreme thermophilic archaea (18). Methanogenic and halophilic archaea have not been conclusively shown to contain these cyclized ether lipids (19). Therefore, the presence of these highly specific compounds is used in the literature as evidence for such an extreme thermophilic environment. Here we report the presence of archaeon-specific C_{40}-ether-bound lipids in particulate organic matter collected from oxic and anoxic marine waters and in a number of marine sediments. These data confirm that archaea are present in a variety of marine environments and suggest that planktonic archaea may constitute an important source of lipids in sediments. The ubiquitous occurrence of these characteristic lipids in the fossil record indicates that planktonic archaea have thrived in marine environments at least for the past 50 million years.

MATERIALS AND METHODS

Samples. The samples studied are described in Table 1. The suspended particle samples from the Cariaco Trench and Black Sea were obtained by in situ filtration through sandwiched 53-μm-pore-size Nitex screens and glass fiber filters (32, 34). The sediment samples from the Black Sea, Cariaco Trench, and Indian Ocean were obtained from several box cores (29, 32, 34). The samples from the Miocene Monterey Formation and Vena del Gesso (Pliocene) are rock samples which were collected from outcrops in the United States and Italy, respectively (15, 20, 26). The Lido Rossello rock samples were collected from the Lido Rossello section in southern Sicily (Italy) by drilling and removing small cores, each with a fixed diameter of 2.5 cm, from the outcrop. In order to collect fresh rock samples the outcrops were cleaned by removal of 0.5 to 1 m of weathered surface.

Extraction and separation. The samples from the Indian Ocean, Lido Rossello section, Monterey Formation, and Vena del Gesso were extracted with dichloromethane-methanol (7:1, vol/vol) and chromatographically separated into fractions of different polarities by using Al_2O_3 as stationary phase (21, 25). The so-called polar fraction, containing the ether lipids, was refluxed in 56% (wt/wt) HI (in H_2O) to cleave the ether bonds; subsequently, the alkyliodides formed were reduced to hydrocarbons with $LiAlH_4$ (21).

The Cariaco Trench and Black Sea suspended particulate organic matter (SPM) and sediment samples were extracted in dichloromethane-methanol (2:1, vol/vol) and chromatographically separated on SiO_2 (5% deactivated with distilled water) into fractions of different polarities by using increasing concentrations of ethyl acetate in hexane and finally methanol. The fraction eluted with methanol was saponified, and the neutrals were refluxed in 57% (wt/wt) HI (in H_2O) for 4 h to cleave the ether bonds. The alkyliodides formed were reduced to hydrocarbons with $LiAlH_4$ in tetrahydrofuran. More details on the analytical methods can be found elsewhere (32).

Gas chromatography. The hydrocarbons released from the Indian Ocean, Lido Rossello section, Monterey Formation, and Vena del Gesso samples were analyzed by gas chromatography (Carlo Erba 8600, equipped with a J&W DB-5 capillary column [0.25-mm internal diameter; 0.25-μm film thickness], or Carlo Erba 5300, equipped with a CP-Sil 5 CB column [0.32-mm internal diameter; 0.12-μm film thickness]) with He as carrier gas by using programmed temperature increases from 70 to 130°C at 20°C min^{-1} and then from 130 to 320°C at 4°C min^{-1}; the temperature was held at 320°C for 20 min. The hydrocarbons released from the Cariaco Trench and Black Sea samples were also analyzed by gas chromatography (Carlo Erba Fractovap 4160 on a DB-5 column [0.25-mm internal diameter; 0.15-μm film thickness]) with H_2 as carrier gas by using programmed temperature increases from 80 to 320°C at 3°C min^{-1}; the temperature was held at 320°C for 20 min.

Gas chromatography-mass spectrometry. The samples were analyzed by gas chromatography-mass spectrometry with an HP 5890 Series II gas chromatograph (with a CP-Sil 5 CB column as described above) coupled to a VG Autospec Q Ultima mass spectrometer (cycle time, 1.7 s; resolution, 1,000) (NIOZ) or an HP 5890 II gas chromatograph coupled to a Finnigan Incos 50 mass spectrometer (SKIO).

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TABLE 1. Samples used in this study

Sample (water depth[a])	Location	Sample type	Depth (m)[b]	Oxicity	Age	Concn (mg/g of organic C)
Cariaco Trench	10°40′N, 65°30′W	SPM	10–250	Oxic	Contemporary	0.1
Cariaco Trench	10°40′N, 65°30′W	SPM	350–1,050	Anoxic	Contemporary	0.4
Cariaco Trench (1,400 m; under anoxic zone)	10°40′N, 65°30′W	Sediment	0–0.01	Anoxic	Contemporary	0.14
Black Sea	43°10′N, 32°00′W	SPM	10–70	Oxic	Contemporary	0.004
Black Sea	43°10′N, 32°00′W	SPM	700–2,000	Anoxic	Contemporary	0.0007
Black Sea (2,200 m; under anoxic zone)	43°10′N, 32°00′W	Sediment	0–0.02	Anoxic	Contemporary	0.1
Black Sea (2,200 m; under anoxic zone)	41°39′N, 30°44′E	Sediment	Unit 2[c]	Anoxic	Recent	0.3
Indian Ocean NIOP 921 (455 m; in OMZ)	16°04′23″N, 52°36′28″E	Sediment	0–0.009	Suboxic	Recent	1.9
Indian Ocean NIOP 921 (455 m; in OMZ)	16°04′23″N, 52°36′28″E	Sediment	0.05–0.08	Suboxic	Recent	0.5
Indian Ocean NIOP 451 (542 m; in OMZ)	23°40′53″N, 66°02′97″E	Sediment	0–0.005	Suboxic	Recent	1.5
Indian Ocean NIOP 464 (1,511 m; below OMZ)	22°15′00″N, 63°34′70″E	Sediment	0–0.005	Oxic	Recent	0.8
Indian Ocean NIOP 453 (1,556 m; below OMZ)	23°15′30″N, 65°44′50″E	Sediment	0–0.01	Oxic	Recent	0.8
Indian Ocean NIOP 458 (3,000 m; below OMZ)	21°59′07″N, 63°48′08″E	Sediment	0–0.01	Oxic	Recent	0.3
Lido Rossello	n.a.[d]	Sediment	n.a.	n.a.	Pliocene	9.5
Vena del Gesso	n.a.	Sediment	n.a.	n.a.	Miocene	n.d.[e]
Monterey Formation	n.a.	Sediment	n.a.	n.a.	Miocene[f]	0–0.3

[a] Where applicable. OMZ, oxygen minimum zone.
[b] Depth of water for SPM samples; depth of sediment for sediment samples.
[c] Unit 2, see reference 32.
[d] n.a., not applicable.
[e] n.d., not determined.
[f] 8 to 18 million years.

Isotope-ratio-monotoring gas chromatography-mass spectrometry. Isotopic compositions of individual compounds were determined by using a Finnigan DELTA-C irm-GC-MS system with similar chromatographic conditions. Isotopic values were calculated by integrating the mass 44, 45, and 46 ion currents of the peaks produced by combustion of the chromatographically separated compounds and that of CO_2 spikes produced by admitting CO_2 with a known ^{13}C content at regular intervals into the mass spectrometer. Values reported are averages of at least two analyses.

RESULTS AND DISCUSSION

In our study of marine water column SPM and sediments, we treated extracts with HI-LiAlH$_4$ to cleave ether bonds in order to release ether-bound compounds. The released fractions were dominated by four components possessing a C_{40} biphytane carbon skeleton and containing 0 to 3 cyclopentane rings (Fig. 1 and 2). In some cases these compounds were found to be more abundant in sediments than lipids known to be derived from eukaryotes, such as dinosterol (2) and long-chain alkenones (30) (Fig. 1). The biphytane carbon skeleton with and without cyclopentane rings is found exclusively in archaea (7, 12, 13). Three of the four compounds (compounds 0 [$C_{40:0cy}$], 1 [$C_{40:1cy}$], and 2 [$C_{40:2cy}$] [Fig. 1]) were identified by coinjection of biphytanes obtained by HI-LiAlH$_4$ treatment of ether lipids from the extreme thermophilic archaeon *Sulfolobus solfataricus* (8). The position of one of the cyclopentane rings in the tricyclic C_{40} biphytane ($C_{40:3cy}$) is different from the $C_{40:3cy}$ biosynthesized by *Sulfolobus* and other archaea. The proposed structure of this $C_{40:3cy}$ (Fig. 1) is based on interpretation of its mass spectrum and that of the dimethylthioether derivative formed after quenching of the alkyliodide obtained from HI treatment of the ether lipids with NaSCH$_3$. A compound with this structure has yet to be identified in any archaeon. However, its structure and occurrence as an ether-bound compound indicate an archaeal origin. The ^{13}C content of the $C_{40:3cy}$ carbon skeleton (Fig. 2) is, in most cases, identical to that of compounds with one and two cyclopentane rings, further suggesting a common origin.

Qualitative distributions of the four C_{40} ether lipids were remarkably similar considering the variety of oxic and anoxic water columns and Recent and ancient sedimentary environments in which the ether-bound lipids were present (Fig. 2).

The $C_{40:1cy}$ compound was consistently the least abundant of the four compounds. Concentrations of ether lipids were quite variable. The sediments contained the highest concentrations, but the concentrations in the SPM samples should be considered minimum values since the 0.7-μm-pore-size filter used in SPM sampling (32) does not efficiently collect archaeal cells unless they are associated with larger aggregates (4a). Furthermore, degradation of labile organic carbon (e.g., carbohydrates and proteins) present in the SPM samples during sedimentation and in the sediment surface layers probably significantly enhances the relative concentration of the selectively preserved ether lipids. Thus, it is unlikely that the higher concentrations in the sediments represent additional input of ether lipids; rather, it is more a reflection of their greater stability.

The stable carbon isotopic compositions of the C_{40} compounds provide insight into the pathways of carbon in archaea. δ-^{13}C values for individual C_{40} lipids ranged from -20 to $-23‰$ (Fig. 2). Within a sample, δ-^{13}C values for individual C_{40} lipids were virtually identical (Fig. 2) and were quite uniform between samples (see Fig. 3 for the $C_{40:3cy}$ compound). This observation is again remarkable considering the different environmental settings and ages from which these compounds were obtained. For comparison, δ-^{13}C values of lipids derived from photosynthetic autotrophs living in the photic zones of the same environments (cholestane derived from cholesterol [25] and lycopane [33]) are also relatively constant, between ca. -24 and $-27‰$ (Fig. 2), although they are 4 to 5‰ depleted in ^{13}C relative to the ether lipid compounds. The relatively constant offset between ^{13}C contents of general algal biomarkers and the archaeal $C_{40:3cy}$ could be explained in two ways. First, the archaea utilize dissolved inorganic carbon as their carbon source, but their biosynthetic pathway may discriminate less against ^{13}C than do algae which use the enzyme Rubisco to fix CO_2 (13). Alternatively, the archaea obtain carbon and energy from low-molecular-weight organic substrates (such as acetate and methylated amines) produced by decomposition of particulate or dissolved organic matter originally derived from algal biomass. Carbohydrate and protein are major biochemicals in algae and are typically 4 to 5‰ enriched in ^{13}C relative to lipids (6). Heterotrophic uptake of algal carbohydrate and
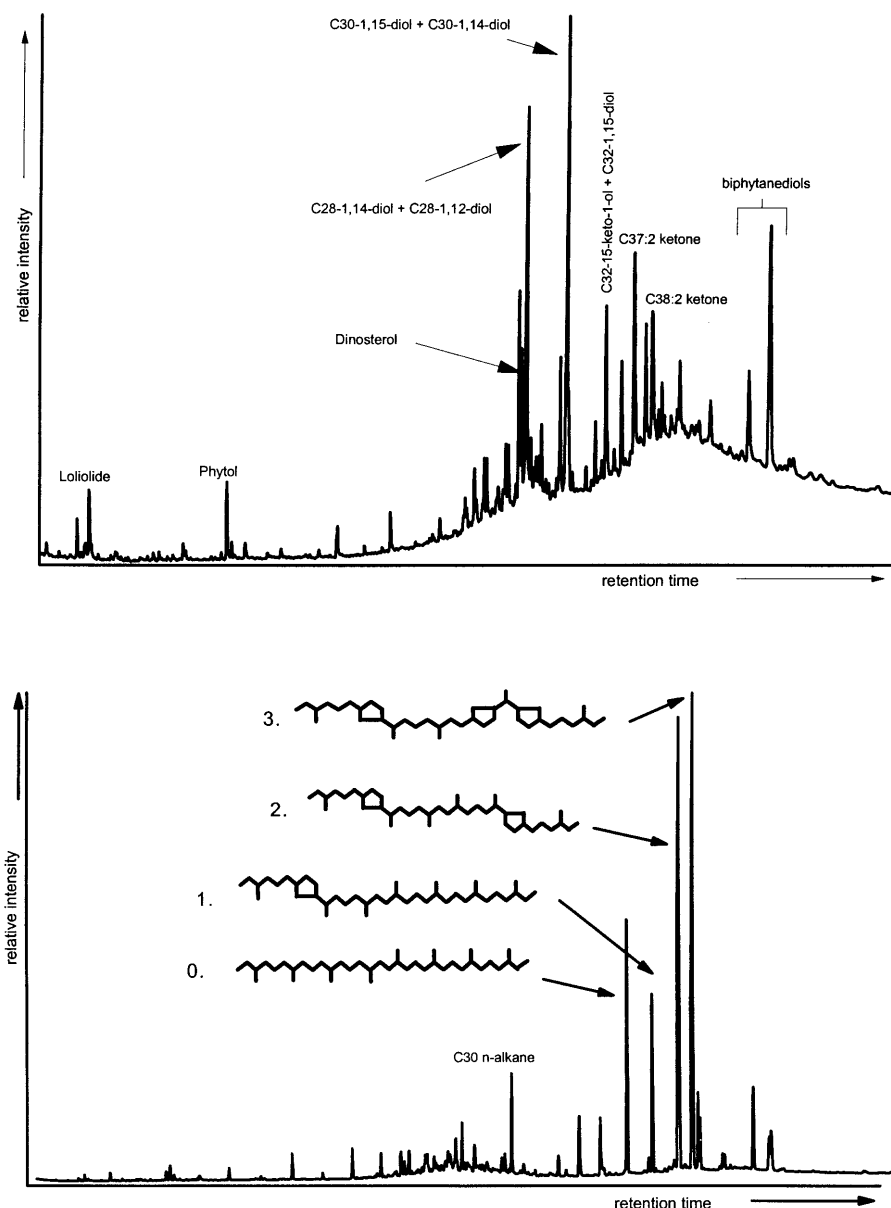
FIG. 1. (Top) Gas chromatogram of polar lipids of maltenes from organic extract of Indian Ocean sediment sample NIOP 451, which contained compounds derived from dinoflagellates (dinosterol [2]), eustigmatophytes (C_{30} diols [31]), and prymnesiophytes (C_{37} and C_{38} alkenones [30]). (Bottom) Gas chromatogram of hydrocarbons obtained after treatment of the polar fraction of maltenes with HI-LiAlH$_4$. Note that the C_{30} n-alkane, derived from C_{30}-alkane-1,15-diol, is only a minor compound compared to the biphytanes.

protein-derived decomposition products would lead to minimal isotopic fractionation and similar isotopic compositions between algal carbon sources and archaeal biosynthetic products (6). At present we cannot differentiate between these possibilities.

The distributions in particulate matter from both oxic and anoxic marine water columns (Fig. 2) of lipids whose occurrence is thought to be restricted to archaea is striking since these organisms generally have been thought to predominate in extreme environments (28). On the other hand, rRNA analyses of marine bacterioplankton (4, 5, 11, 12) suggest that archaea are common in marine waters (up to 34% of prokaryotic biomass [4, 5]). The rRNA gene sequences of the planktonic archaea (analyzed in different settings and size fractions than in our study) are not identical to rRNA of known archaea, but phylogenetic analyses indicate that the closest characterized relatives are extreme thermophiles or methanogens. Although hyperthermophilic archaea can survive low-temperature oxic conditions (16) and could be advected from hydrothermal regions of the ocean (14), gene sequences of planktonic archaea are sufficiently different from those of thermophiles to suggest that planktonic archaea are in fact not thermophiles but may have diverged from thermophilic archaea early in evolution (4, 5, 11, 12). Gene sequences of the archaeal bacterioplankton are also distinct from those of known methanogens, but it is not possible to rule out the possibility that some water column archaea are methanogens, inhabiting transient anoxic microzones (17) in otherwise oxic seawaters.
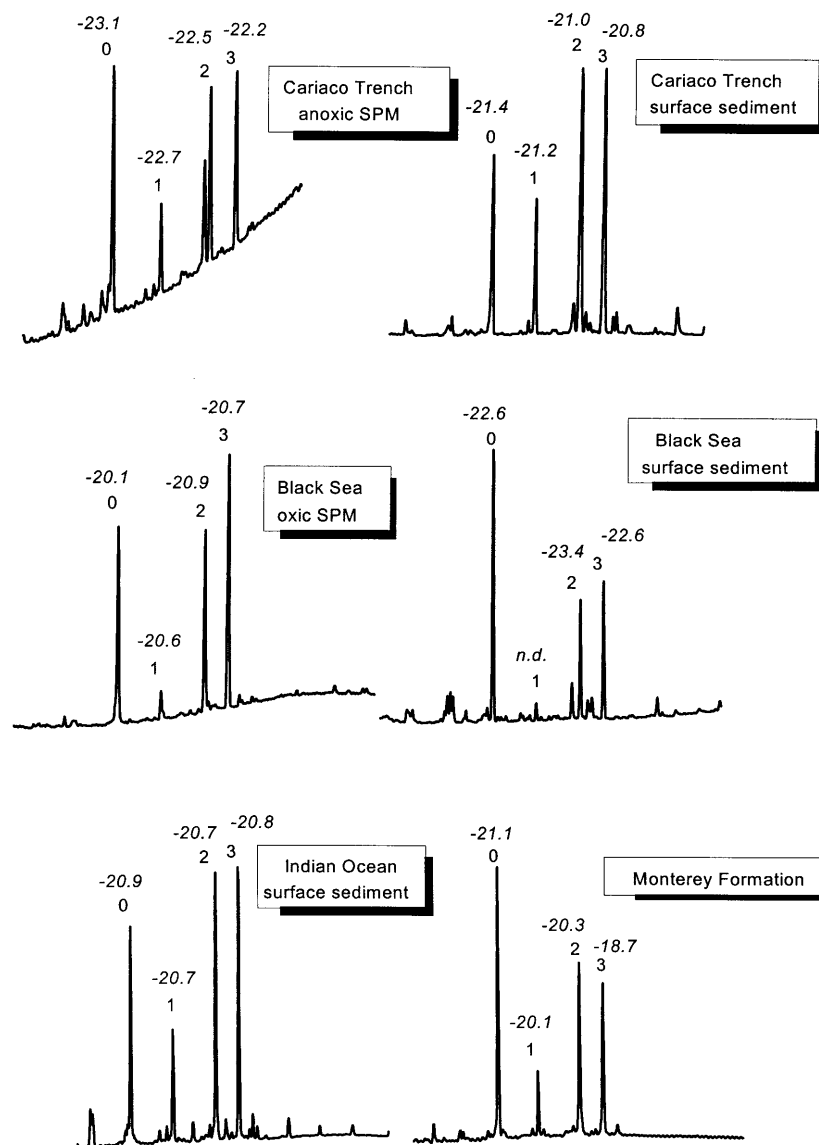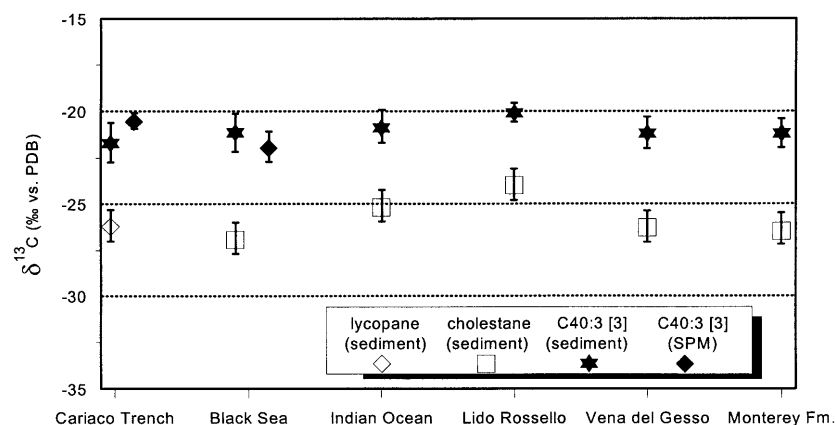
FIG. 2. Partial gas chromatograms showing the distributions and isotopic compositions (in ‰) of acyclic and cyclic biphytanes in sediment and particulate samples from the Black Sea, Cariaco Trench, Arabian Sea (NIOP), and Monterey Formation (see also Table 1). Particulate organic matter samples and sediments were collected in the Black Sea, the Cariaco Trench (offshore of Venezuela [32, 34] [Table 1]), and the Indian Ocean (29), and outcrop samples were obtained from the Pliocene Lido Rosello, the Miocene Vena del Gesso (20, 21), and the Monterey Formation (25, 26) (Table 1). n.d., not determined.

These molecular biological observations of the presence of archaea in surface ocean waters are now complemented by our ether lipid distributions. Acyclic isoprenoid ether lipids, including the $C_{40:0cy}$ biphytane reported here, occur in a number of thermophiles and methanogens. Cyclic $C_{40:1cy}$, $C_{40:2cy}$, and $C_{40:3cy}$ biphytanes appear to be restricted to extreme thermophiles (7). The biosynthesis of cyclic biphytanes in methanogens is uncertain: $C_{40:2cy}$ has been reported to occur in the methanogen *Methanosarcina barkeri* (9), but subsequent investigations (references 23 and 27 and this study) failed to detect cyclic biphytanes. Furthermore, current investigations of the ether lipids in a number of common marine methanogens have failed to detect cyclic biphytanes (26a). The $C_{40:3cy}$ lipid in our SPM and sediment samples is apparently different from the $C_{40:3cy}$ commonly biosynthesized by thermophiles (e.g., *Sulfolobus* organisms), suggesting that the archaea bio-

synthesizing this carbon skeleton are different from well studied cultured thermophiles.

Two aspects of our results indicate that planktonic archaea may be an important source of the cyclic biphytanes in our samples, including the sediments. First, the occurrence of these compounds in oxic SPM samples strongly suggests a bacterioplanktonic origin. Second, carbon isotopic compositions of cyclic biphytanes in both water column and sediment samples from a variety of environments and over a large range of ages are very similar and are consistently offset relative to other planktonic biomarkers. While archaea undoubtedly inhabit sedimentary environments (for example, see reference 22), it is possible that their ether lipid composition and stable carbon isotopic compositions would be distinct from those of euphotic-zone species since concentrations and δ-^{13}C values of dissolved inorganic carbon and/or low-molecular-weight sub-

FIG. 3. Graph showing the stable isotopic compositions of the $C_{40:3cy}$ biphytane (compound 3 in Fig. 1) and lipids (cholestane, or, in the case of the Cariaco Trench, lycopane) indicative of photosynthetic autotrophs (25, 33). PDB, Pee Dee Belemnite.

strates in sedimentary environments would be quite different from those of surface waters (1). The abundance of cyclic ether lipids in oxic water column SPM and oxic sediments also argues against methanogenic archaea as an important component of the archaea contributing ether lipids to our samples. It remains to be seen, however, whether the planktonic archaea detected by rRNA analyses are those species producing the ether lipids we have detected.

In contrast to acyl-based membrane lipids in eubacteria and eukaryotes which are highly susceptible to phospholipases released by other organisms, the unique alkyl-ether structures of archaeal membrane lipids impart stability over the range of environmental conditions in which archaea thrive (18). This stability also results in enhanced preservation of ether lipids in sediments, whereas both the archaeal rRNA and acyl-ester lipids derived from other organisms are quickly degraded. Thus, the presence of specific C_{40} ether lipids with specific isotopic compositions in sediments indicates inputs from planktonic archaea to past depositional environments. Carbon isotope analyses of fossil sedimentary C_{40} ether lipids (Fig. 2) also suggest that planktonic archaea in the past apparently occupied the same ecological niches as today's planktonic archaea. Cyclic ether lipids have been found in sediments ranging from Recent to at least Eocene (50 million years BP) (3, 21), indicating that planktonic archaea have existed at least since these times. Thus, a further search for the cyclic biphytanes in the geological record, in combination with their carbon isotopic composition, may provide a clue to the evolutionary origin of marine planktonic archaea.

ACKNOWLEDGMENTS

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